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IDENTIFICATION OF ACETYLCHOLINE, 5-HYDROXYTRYPTAMINE, HISTAMINE, AND A NEW KININ IN HORNET VENOM (*V. CRABRO*)

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The venom of the common wasp, *Vespa vulgaris*, contains 5-hydroxytryptamine (5-HT), histamine and a kinin (Jaques & Schachter, 1954; Holdstock, Mathias & Schachter, 1957). The present experiments demonstrate that, like that of the wasp, the venom of the European hornet, *V. crabro*, contains 5-HT and histamine. It differs from that of the wasp, however, in that it contains high concentrations of ACh and also a kinin which is not identical with the one in wasp venom.

In recent years evidence has accumulated of the existence in nature of pharmacologically active choline esters other than ACh, such as propionylcholine (PrCh) in ox spleen (Banister, Whittaker & Wijesundera, 1953), urocanylcholine (Murexine) and β-β-dimethylacrylylcholine (DMAC) in the hypobranchial glands of certain molluscs (Erspamer & Benati, 1953; Keyl, Michaelson & Whittaker, 1957). An active ester of choline, closely resembling or identical with DMAC, has also been found in the cervical (defensive) gland of the Garden Tiger moth, *Arctia caja* (Bisset, Frazer, Rothschild & Schachter, 1960). The choline ester in hornet venom was therefore carefully examined by chemical and pharmacological tests and was identified as ACh.

The kinin in hornet venom is classified as such because it contracts the isolated rat uterus and guinea-pig ileum, relaxes the rat duodenum, and lowers the arterial blood pressure of the rabbit. It has been distinguished from oxytocin, wasp kinin and bradykinin, but closely resembles the latter.

A preliminary report of this work has been communicated to the Physiological Society (Bhoola, Calle & Schachter, 1960).

METHODS

Hornet venom. A nest of live hornets was kindly given to us by Mr R. L. Ford, F.R.E.S. The hornets were frozen at $-10°$ C, then thawed, and the venom apparatus of the females removed by pulling gently on the sting with fine forceps. The venom sacs were dissected free of adjacent tissue, accessory glands and stinging apparatus, and dried *in vacuo* over P_2O_5.

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Extracts. Dried venom sacs (1–5 at a time) were extracted five times with hot ethyl alcohol (95 %). The first extraction was with 1·0, 0·5 or 0·25 ml. (depending on the number of sacs extracted), and the subsequent ones with 0·25 ml. The extract and residue were reduced to dryness *in vacuo*. For assays without preliminary chromatography sacs were extracted with boiling, acidified (HCl) Tyrode solution (pH 3–4) and centrifuged, and the supernatant solution neutralized and tested.

Isolated organ preparations. The isolated guinea-pig ileum, rat duodenum or rat uterus was suspended in a 17 ml. bath in Mg-free Tyrode solution (%: NaCl, 0·8; KCl, 0·02; $CaCl_2$, 0·02; NaH_2PO_4, 0·005; $NaHCO_3$, 0·1; glucose, 0·1) and contractions were recorded with a frontal lever writing on a smoked drum. The bath temperature was adjusted to 35, 30 and 26–28° C for the guinea-pig ileum, rat duodenum and rat uterus respectively. Atropine (10^{-8} g/ml.), mepyramine (10^{-8} g/ml.) and lysergic acid diethylamide (LSD; 10^{-7} g/ml.) were added to the bath when required.

The rectus abdominis muscle of the frog (*Rana temporaria*) was suspended in an 8 ml. bath containing eserinized (5×10^{-6} g/ml.) Ringer's solution at room temperature. Acetone was added to the bath before each test to give a concentration of 1:4000 (v/v); this was found to increase the sensitivity to ACh five- to tenfold. D-Tubocurarine (10^{-6} g/ml.) was added when required.

Blood pressure. Rabbits were anaesthetized with a solution of sodium phenobarbitone (60 mg/kg) and urethane (500 mg/kg) (= 2 ml./kg) injected intravenously, and blood pressure was recorded from the carotid artery with a mercury manometer. Test substances were injected through a cannula in the external jugular vein and washed into the circulation with a constant-volume injection apparatus.

Paper chromatography. Ethyl alcohol (95 %) extracts of 1–6 venom sacs were dried, redissolved in 50 % ethyl alcohol and applied as single spots (equivalent of 0·5–1·5 mg dry venom sac) or as a 5 cm strip (equivalent of 10 mg dry venom sac) on the paper. Markers of acetyl-, butyryl-, and propionylcholine, choline, histamine and 5-HT (25 or 50 μg) were also applied as spots. The ethyl alcohol-insoluble material in the sac was dissolved in a small volume of distilled water, centrifuged, and applied as a 5 cm strip (equivalent of 10 mg dry sac).

Ascending chromatograms were run overnight at room temperature (16–20° C) on Whatman No. 1 paper. The solvents used were: *n*-butanol:ethanol:acetic acid:water (8:2:1:3) (Augustinsson & Grahn, 1953); *n*-butanol:acetic acid:water (4:1:5) (Partridge, 1948); *iso*propanol:ammonia:water (20:1:1) (Smith, 1958); *n*-propanol:formic acid:water (8:1:1), *n*-butanol:water (9:1), and *n*-propanol:water (9:1) (Whittaker & Wijesundera, 1952); *iso*propanol:0·1 N-HCl (7:3) (Lembeck, 1954), and ethanol:0·2 N-HCl (1:1) (Holdstock *et al.* 1957).

Chromatograms were examined under ultra-violet light and fluorescent or quenching areas outlined. Development was by (*a*) Iodine vapour, (*b*) Pauly's reagent: diazotized 1 % (w/v) sulphanilamide in *n*-butanol followed by 50 % saturated sodium carbonate, (*c*) Ehrlich's reagent: 2 % (w/v) *p*-dimethylaminobenzaldehyde in 5 % hydrochloric acid, (*d*) Ninhydrin-acetic acid: 0·25 % (w/v) ninhydrin in acetone (9 parts) in acetic acid (1 part); paper examined under U.V. light (Jepson & Stevens, 1953), (*e*) Ninhydrin-pyridine: 0·25 % (w/v) ninhydrin in acetone plus several drops of pyridine; paper examined under U.V. light, (*f*) Shepherd's reagent: 0·1 % potassium dichromate in 35–40 % formaldehyde, (9/1, v/v); after spraying, heat for 5 min at 110° C and examine under U.V. light (Shepherd & West, 1953), (*g*) Phosphomolybdic acid-stannous chloride: 2 % (w/v) phosphomolybdic acid in ethanol/chloroform (1/1, v/v) followed by 10 % (w/v) stannous chloride in 3 N-hydrochloric acid (cf. Block, Durrum & Zweig, 1958).

For bioassay the paper was cut into horizontal strips beginning 1 cm on either side of the origin, and up to the solvent front as strips 1 or 2 cm wide. Each section was eluted with 1·5–3·0 ml. warm Tyrode solution and 0·05–0·5 ml. eluate was tested on the isolated test preparation.

Ultra-violet absorption spectrum. The area on chromatograms (*n*-propanol:formic acid: water) which corresponded in R_F value and colour reactions to 5-HT was eluted in 3·0 ml. water and its absorption spectrum read in 1·0 cm quartz cells in the Hilger 'Uvispek' against a blank eluted from an adjacent area of the chromatogram exposed only to the solvent.

Drugs and other reagents. Histamine acid phosphate, 5-HT creatinine sulphate, choline, acetyl-, and propionylcholine chloride, butyrylcholine perchlorate, eserine alkaloid, atropine sulphate, mepyramine maleate and D-tubocurarine chloride were the drugs used. The weights of histamine and 5-HT are expressed as base.

Trypsin and chymotrypsin were crystalline preparations (Armour).

Bradykinin was prepared by the action of crystalline trypsin on heated ox serum globulin (Holdstock *et al.* 1957) and contained 140–900 units/mg; wasp kinin was prepared from wasp venom (Holdstock *et al.* 1957).

RESULTS

Initial attempts at bioassay and specific identification of substances in hornet venom sacs indicated that four or more distinct activities were present. Even with specific antagonists of cholinesters, of histamine or of 5-HT, it was not possible to be confident of quantitative assay of more than two substances in the extract. For example, in tests on the isolated guinea-pig ileum it was evident that the major activity could be assayed and antagonized by atropine; similarly, the histamine in the venom could then be assayed on the same preparation in the presence of atropine and its action antagonized by mepyramine. The assay of the remaining activities was difficult, however, because the concentration of ACh-like activity in the extracts was so great, that increased concentrations of atropine were required in attempts to assay the atropine-resistant activity. It was therefore necessary first to separate the active substances by paper chromatography.

After unsuccessful attempts at separation by chromatography in several solvents, we found that a distinct separation of three substances, chromatographically and pharmacologically identical with ACh, 5-HT and histamine respectively, was possible by using *n*-propanol:formic acid: water as a solvent (Fig. 1). However, since the area on this chromatogram corresponding to 5-HT in R_F value and colour reactions was only partially referable to 5-HT in pharmacological tests (approximately one-half of the activity of the eluate was antagonized by LSD), this eluate was re-chromatographed. It was eventually successfully resolved into two components, one identical with 5-HT, the other having the properties of a kinin. The most effective solvent for this purpose was ethanol:0·2N-HCl (1:1).

Acetylcholine

Activity of extracts on isolated guinea-pig ileum. The ACh-like activity in extracts (0·9% NaCl + HCl; pH, 3–4) could be assayed on the guinea-pig ileum because the high concentrations present permitted the assay of small amounts of extract, the activity of which was completely antagonized

by atropine in low concentrations (10^{-8} g/ml). The ACh-equivalent concentrations in 4 dry venom sacs tested in this way were 18, 33, 40 and 50 mg/g respectively. One sac extracted with 95 % ethyl alcohol contained 18 mg/g (Table 1).

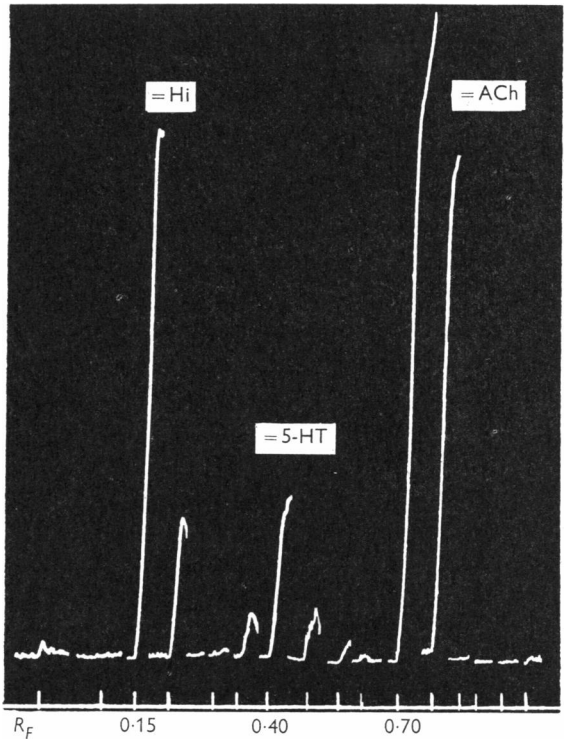

Fig. 1. Contractions of guinea-pig ileum to eluates from a chromatogram (*n*-propanol:formic acid:water) of alcohol extract of hornet venom sac. Three separate activities are eluted which correspond to histamine (Hi), 5-HT and ACh in R_F values, colour reactions and specific antagonism. The eluate corresponding to 5-HT was, however, incompletely antagonized by LSD when tested on the isolated rat uterus, and in fact also contains a kinin which can be separated in another solvent (see Fig. 5).

TABLE 1. Concentrations (mg/g dry weight) of ACh, histamine and 5-HT in hornet venom sacs

Weight of sac (mg)	ACh	Histamine	5-HT	Method
2·8	50	30	—	Acid extract
2·0	33	17	—	
2·2	40	20	—	
3·0	18	14	—	
3·0	22	18	—	Ethanol extract
2·7	10	3	7	Chromatogram eluate
2·8	—	—	15	
8·0 (3 sacs)	—	—	19	

Chromatography, elution and bioassay. As there is now evidence that different, active cholinesters exist in nature (see Discussion) it was necessary to identify the ACh-like substance more specifically. Alcoholic extracts of 1–3 venom sacs were therefore applied as 3–6 spots about 2·5 cm apart on the same paper and chromatographed. After chromatography in *n*-propanol:formic acid:water solvent some of the chromatograms were sprayed with iodine or phosphotungstic acid–stannous chloride reagent, and another similar one was cut into small horizontal strips (see Methods), which were eluted. One portion of each eluate was tested on the isolated guinea-pig ileum and another on the frog rectus abdominis muscle. In every case the eluate which corresponded to ACh in R_F value contracted both the guinea-pig ileum and the frog rectus muscle, the former effect being antagonized by atropine (10^{-8} g/ml.) and the latter by D-tubocurarine (10^{-6} g/ml.). Although the eluates of several distinct areas on the chromatogram contracted the guinea-pig ileum (Fig. 1), the activity of only one was blocked by atropine, and only it contracted the frog rectus muscle (Fig. 2).

To obtain further evidence of the identity of this substance with ACh 10 hornet venom sacs weighing 21·6 mg were extracted with ethyl alcohol and chromatographed as a narrow strip 2·5 cm long in *n*-propanol:formic acid:water solvent, together with separate marker spots of the same extract and of ACh. The latter were developed with iodine, and the area of the main venom strip corresponding to these in R_F value and in colour reaction was eluted with 50 % ethyl alcohol. The eluate was reduced in volume *in vacuo* and applied as single spots on five separate chromatograms and run overnight in (*a*) *n*-butanol:water, (*b*) *n*-propanol:water, (*c*) *n*-butanol:acetic acid:water, (*d*) *n*-propanol:formic acid:water, and (*e*) ethyl alcohol:hydrochloric acid. Markers of choline, ACh, PrCh, and BuCh were also placed on each paper. On development with iodine the re-chromatographed venom eluate developed in each case at one distinct spot only, closely agreeing with that of the ACh marker (Fig. 3).

Parallel assay. Parallel pharmacological tests with the active eluate from a chromatogram (*n*-propanol:formic acid:water) corresponding to ACh in R_F value and iodine reaction, gave an index of discrimination which did not deviate significantly from 1·0 in tests on the isolated guinea-pig ileum, frog rectus muscle, and blood pressure of the rabbit; the hypotensive effect of the eluate was also blocked by intravenous injection of atropine (Fig. 4).

Table 2 shows the R_F values obtained for ACh, histamine and 5-HT, and indicates clearly that with the choice of appropriate solvents a ready separation of these substances can be obtained.

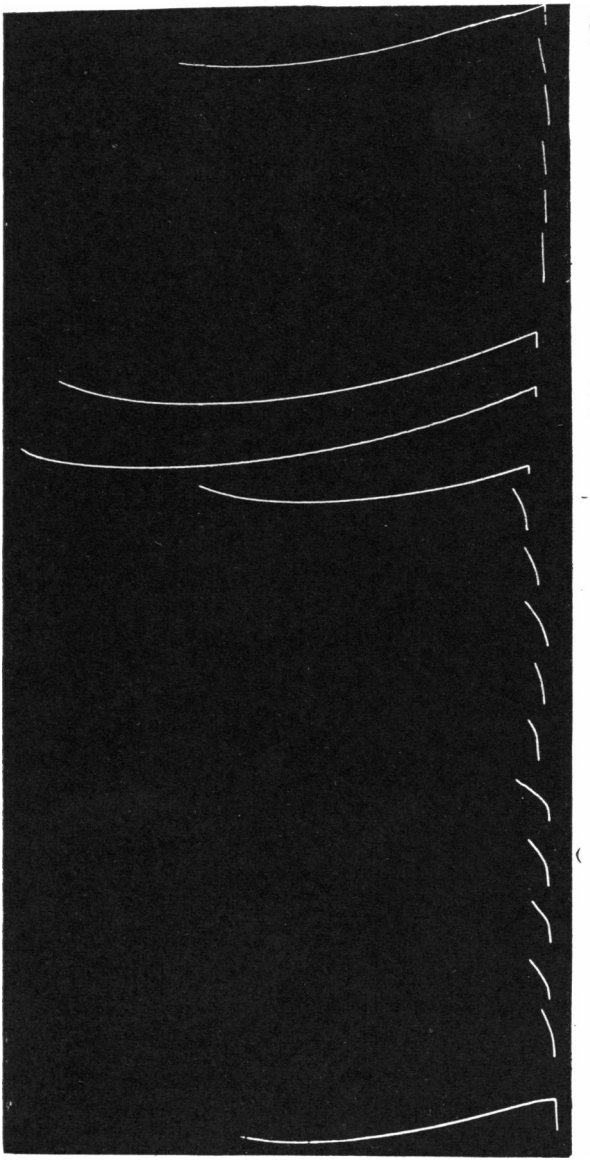

Fig. 2. Contractions of isolated frog rectus muscle (eserine 5×10^{-6} g/ml.; acetone 1/4000 (v/v)) to eluates from a chromatogram (*n*-propanol: formic acid: water) of alcohol extract of hornet venom sac. There is only a single band of activity which coincided with that of the ACh marker.

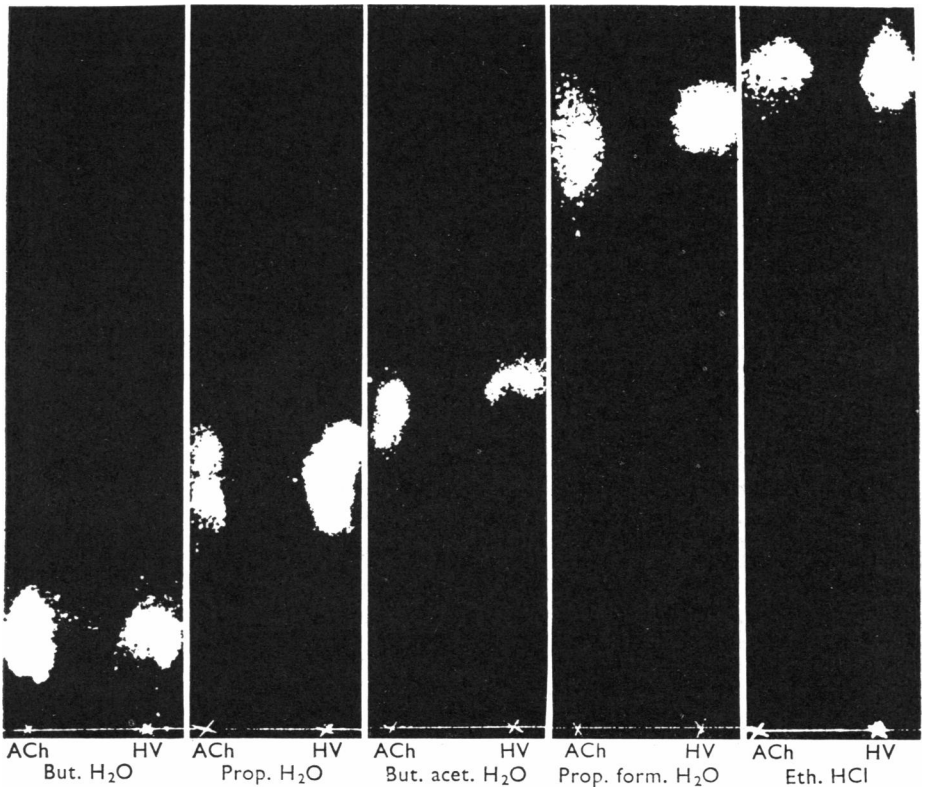

Fig. 3. Chromatographic identification of ACh in hornet venom. The ACh-like substance on a chromatogram (*n*-propanol:formic acid:water) of alcohol extract of hornet venom was eluted and rechromatographed in five different solvents. In each solvent iodine development (above) and eluted pharmacological activity corresponded to that of ACh in R_F value.

Histamine

Effect of extracts on isolated guinea-pig ileum. Acidified saline and ethanol extracts of venom sacs were assayed for histamine on the isolated ileum as for ACh, but in the presence of atropine (10^{-8} g/ml.). The activity assayed was then completely abolished by mepyramine (10^{-8} g/ml.). The histamine concentration was always less than that of ACh, varying from 50 to 80 % of that of ACh (Table 1).

Chromatography. Chromatograms in all solvents regularly yielded an active eluate which corresponded to histamine in R_F value, gave a similar Pauly reaction and caused a contraction of the guinea-pig ileum which was abolished by mepyramine.

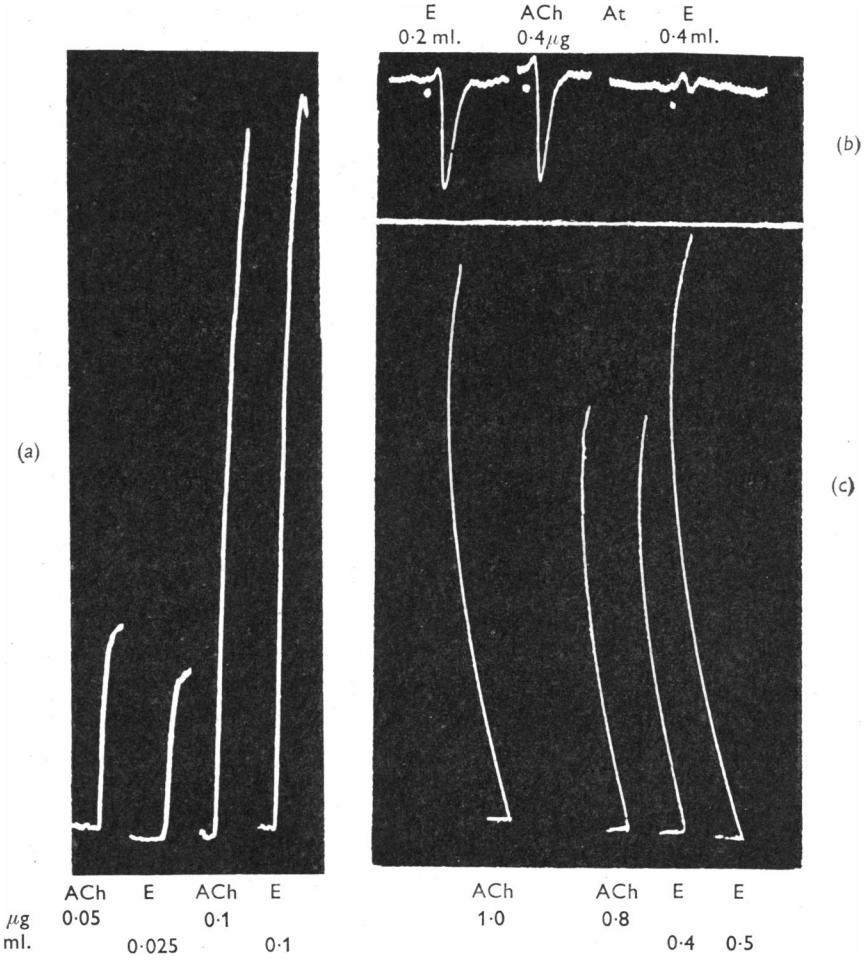

Fig. 4. Quantitative parallel activity of ACh and eluate from chromatogram (*n*-propanol:formic acid:water) of alcoholic extract of hornet venom sac. The eluate corresponded to ACh in colour reactions and in R_F value. E, eluate; At, atropine, 0·2 mg/kg. (*a*) guinea-pig ileum; (*b*) rabbit blood pressure; (*c*) frog rectus muscle.

5-Hydroxytryptamine

It was not possible to assay 5-HT in extracts of venom directly on the guinea-pig ileum because of the high concentrations of the other active substances. Even in the presence of atropine and mepyramine, when increased amounts of venom were tested the high concentrations of ACh and histamine overcame the drug antagonism. Similar difficulties developed with other test preparations. For quantitative assay and more

definite identification of 5-HT it was necessary, therefore, to use chromatography to separate it from the other substances.

Alcoholic extracts of 1–3 glands were divided equally, applied to the paper as several separate spots, and chromatographed in *n*-propanol: formic acid: water. The chromatogram showed a well defined area with the same R_F value as the 5-HT marker; it gave a red Pauly reaction, a bluish-grey colour with Ehrlich's reagent, a positive ninhydrin and ninhydrin-acetic-acid reaction—the latter showing green fluorescence under ultraviolet light and a golden-yellow colour with Shepherd's reagent. Like 5-HT the eluate from this area contracted the guinea-pig ileum (Fig. 1)

TABLE 2. R_F values of ACh, histamine, 5-HT and hornet kinin in different solvents

Solvent	ACh	Histamine	5-HT	Kinin (in ethanol extract)	Kinin (in ethanol insoluble residue)
*iso*Propanol: ammonia: water (20:1:1)	—	0·38	0·45	—	—
n-Butanol: ethanol: acetic acid: water (8:2:1:3)	—	0·21	0·57	—	—
n-Propanol: formic acid: water (8:1:1)	0·64	0·16	0·40	0·40	—
n-Butanol: acetic acid: water (4:1:5)	0·37	—	0·40	0·34	0·35
*iso*Propanol: 0·1 N-HC (7:3)	0·85	—	0·55	0·61	—
Ethanol: 0·2 N-HCl (1:1)	0·63	—	0·56	0·80	0·78
n-Butanol: water (9:1)	0·10	—	—	—	—
n-Propanol: water (9:1)	0·26	—	—	—	—

and the isolated rat uterus, but its activity on the rat uterus was reduced by only about 50 % by LSD. This suggested the presence of an active contaminant with the same R_F value as 5-HT in this solvent. This was confirmed when the LSD-resistant activity of the eluate was completely inactivated by incubation with chymotrypsin (25 μg/ml.) for 10 min (Fig. 5). The separation of the two components was then effected by rechromatographing the eluate in ethanol: HCl, the chymotrypsin-resistant material moving faster than the LSD-sensitive one (Fig. 5). Now only the latter eluate, like 5-HT, reacted with Pauly's reagent and was antagonized completely by LSD.

The concentration of 5-HT in venom was assayed on the rat uterus, using eluates (from *n*-propanol: formic acid: water chromatograms) which corresponded to 5-HT in R_F value and in Pauly reaction, after they had been incubated with chymotrypsin (25 μg/ml.) (Table 1). The concentrations of 5-HT were of the same order as those of histamine (Table 1).

Kinin

The substance contracting the rat uterus and contaminating the 5-HT area on chromatograms of venom, though inactivated rapidly by chymotrypsin (25 μg/ml.) (Fig. 5), was unaffected by trypsin. It thus differed

from wasp kinin which is inactivated by trypsin (Schachter & Thain, 1954), but resembled kallidin or bradykinin (Holdstock *et al.* 1957). It was therefore isolated further, freed from 5-HT, and its properties studied in more detail.

The area on venom chromatograms (*n*-propanol:formic acid:water) which included this substance and the associated 5-HT was eluted and re-chromatographed in several solvents. On testing the eluates re-chromatographed in *n*-butanol:acetic acid:water, activity was found in the area

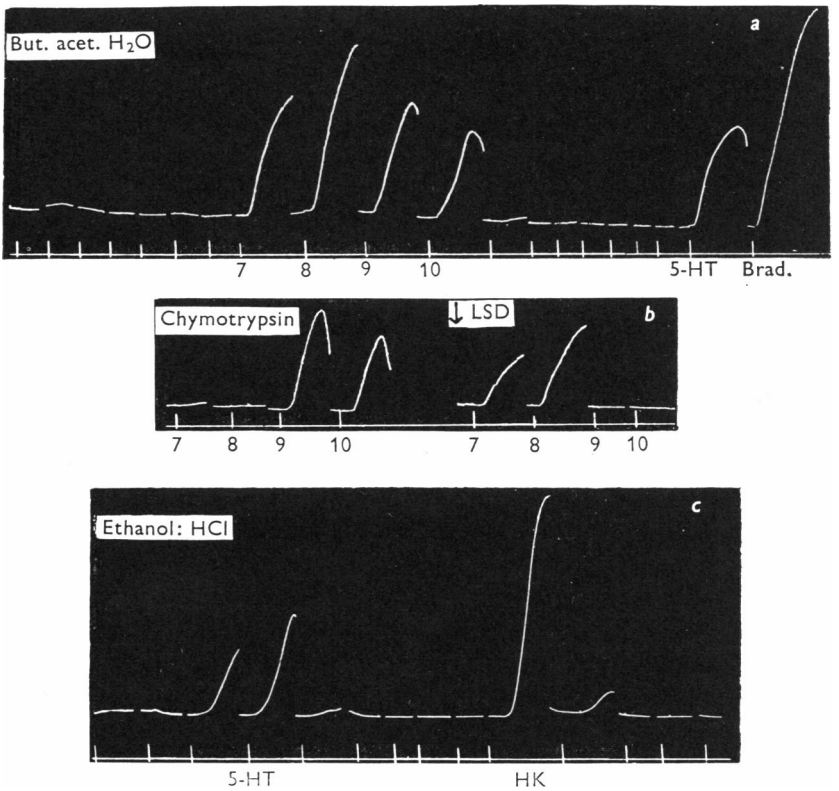

Fig. 5. Separation of 5-HT and kinin (HK) in hornet venom by paper chromatography. Contractions of isolated rat uterus (26–28° C) to chromatogram eluates. (*a*) The eluate corresponding to 5-HT in R_F value and colour reactions on a chromatogram (*n*-propanol:formic acid:water) was eluted and rechromatographed in butanol:acetic acid:water. The eluates of strips 7, 8, 9 and 10 (see Methods) were active, and the types of contraction suggested the presence of two substances inadequately separated. (*b*) The incubation of strips 7–10 with chymotrypsin (20 μg/ml., 10 min) inactivated 7 and 8 only; addition of LSD (10^{-7} g/ml.) to muscle bath antagonizes 9 and 10 only. (*c*) *Rechromatographed* pooled eluates (7, 8, 9 and 10) in ethanol: HCl; the two activities are successfully separated, the slower-moving compound being identical with 5-HT and the other with the kinin (HK).

corresponding to that reacting with Pauly's reagent and with the 5-HT marker, and it was completely antagonized by LSD (10^{-7} g/ml.). An area just behind this one on the chromatogram, but continuous with it, did not develop with Pauly's reagent and was unaffected by LSD, but was rapidly inactivated when incubated with chymotrypsin (Fig. 5). It was apparent therefore that re-chromatographing in this solvent effected a slight but still inadequate separation of 5-HT and the apparent peptides. A similarly inadequate separation was obtained with chromatograms re-run in *iso*-propanol:hydrochloric acid; in this instance 5-HT moved slightly ahead of the other activity.

A successful separation was, however, effected on re-chromatographing in ethanol:HCl. In this instance 5-HT was recovered as a sharp band with an R_F value of 0·56, whereas the LSD-resistant, but chymotrypsin-resistant material, was recovered much higher on the paper, with an R_F value of 0·80 (Fig. 5). The latter was now classified as a kinin since it also produced a slow contraction of the guinea-pig ileum, relaxation of the rat duodenum and a marked lowering of the arterial blood pressure of the rabbit. The amount of venom available did not permit precise parallel assays of this eluate with bradykinin but it was found to be relatively one tenth as active as bradykinin on the guinea-pig ileum.

Pharmacological tests of the alcohol-insoluble residue of venom sacs showed it was free from, or contained only traces of, ACh, histamine or 5-HT. It did, however, still possess much activity when tested on the rat uterus. This was unaffected by LSD, atropine or mepyramine, or by incubation with trypsin, but was rapidly inactivated by chymotrypsin (Fig. 6). The substance was studied in more detail, since the absence of other active substances in the residue permitted a simple and direct analysis. The insoluble residue when assayed against crude bradykinin on the rat uterus contained at least 350 μg bradykinin/g in terms of the pure synthetic substance. (Since, as shown below, the kinin in the alcohol extract is identical with that in the residue, the amount present in the venom is much greater than this.) It also contracted the guinea-pig ileum, relaxed the rat duodenum, and lowered the arterial blood pressure of the rabbit. It behaved as a single substance when chromatographed in *n*-butanol:acetic acid (Fig. 6) and in ethanol:HCl solvents, and had the same R_F values as the kinin in the alcohol extract. Also, a mixture of the two could not be separated by paper chromatography. It seems very likely, therefore, that these two kinins are the same substance, which is partially soluble in 95 % ethyl alcohol when extracted from venom sacs.

Quantitative parallel assay of this kinin against bradykinin showed a very close similarity, but, like the alcohol-soluble kinin, it was relatively only approximately one tenth as active as bradykinin on the guinea-pig

ileum. Despite the similar behaviour of hornet kinin and oxytocin on the rat uterus there were great quantitative discrepancies in their relative activities on this and other preparations (Table 3). Hornet kinin, unlike wasp kinin, was not inactivated by trypsin (Fig. 6); mixtures of the two

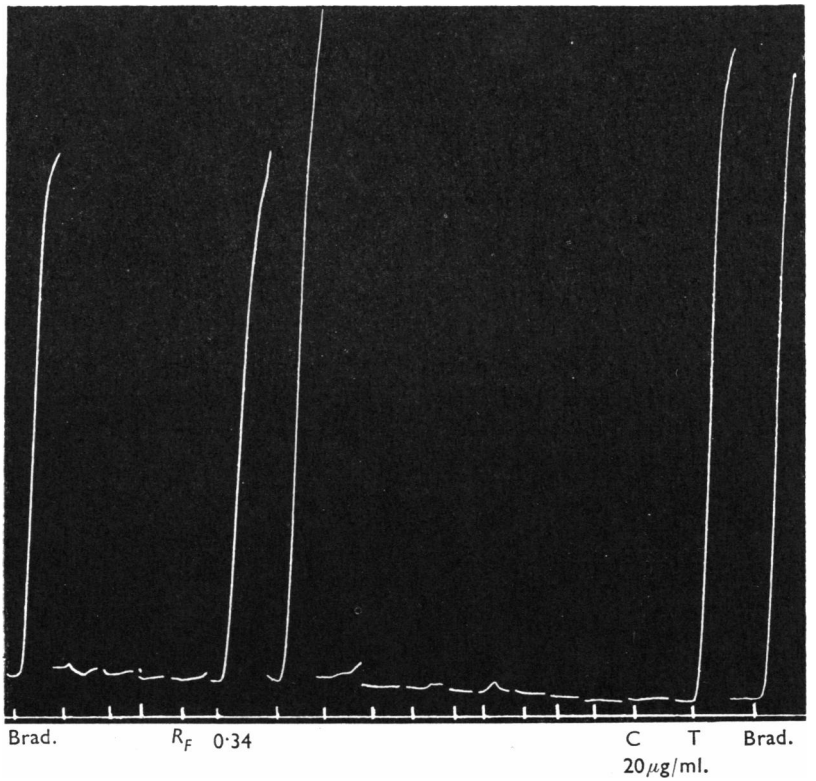

Fig. 6. Paper chromatography of hornet kinin (in alcohol-insoluble residue of venom sac) and its inactivation by chymotrypsin but not by trypsin. Contractions of rat uterus (28° C; atropine and LSD, 10^{-8} and 10^{-7} g/ml.) to eluates from chromatogram (*n*-butanol: acetic acid: water). C and T are incubated mixtures (10 min) of active eluate (R_F, 0·34) with chymotrypsin and trypsin respectively; Brad. = bradykinin, 2 u.

were also readily separated by paper chromatography in *n*-butanol: acetic acid, in which, as shown by Holdstock *et al.* (1957), only wasp kinin remained at the origin (Fig. 7). There was no evidence of wasp kinin in hornet venom since not a trace of activity was found at the origin of chromatograms in this solvent.

The similar pharmacological actions of hornet kinin and bradykinin are shown in Fig. 8; the former is relatively less active on the guinea-pig ileum.

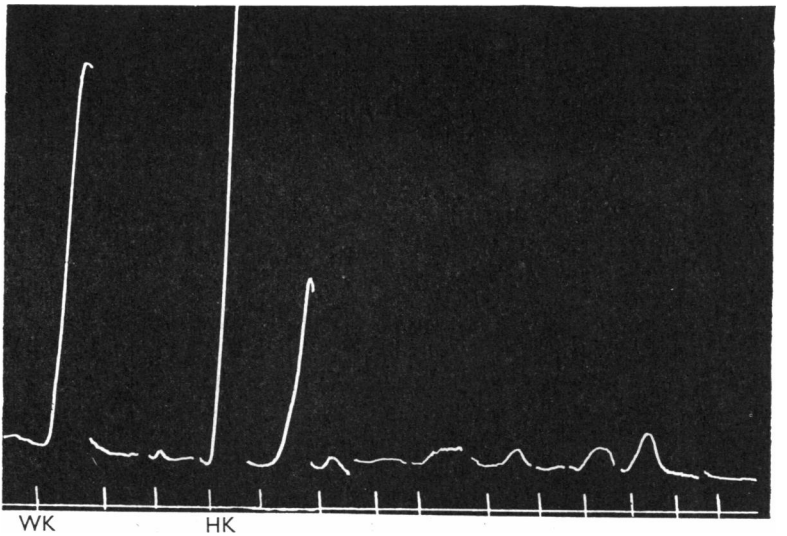

Fig. 7. Separation of wasp kinin (WK) and hornet kinin (HK) by paper chromatography (*n*-butanol: acetic acid: water). Contractions of rat uterus (28° C, atropine 10^{-8} g/ml. and LSD 10^{-7} g/ml.) to eluates from a chromatogram of a mixture of wasp and hornet kinin. One activity appears at R_F value 0 and is wasp kinin; the other at 0·34 and is hornet kinin.

TABLE 3. Bradykinin standard and oxytocin (Syntocinon, Sandoz) equiactive with 1 mg hornet kinin

	Bradykinin (μg)	Oxytocin (m-u.)
Guinea-pig ileum	125	—
Rat uterus	1600	800
Rat duodenum	700	23,000
Rabbit blood pressure	1600	> 85,000 (pressor)
Index of discrimination	12·8	> 100

DISCUSSION

Our experiments demonstrate that hornet venom contains four substances which are known to be among the most pharmacologically active agents present in animal tissues. There is little doubt that these substances in such concentrations, are effective in defence reactions and contribute to the severity of toxic reactions in man. The likelihood that enzymes and other proteins which add to the toxicity are present in the venom is, however, not excluded.

Hornet venom differs in composition from wasp venom in that it contains ACh and also a kinin which differs from that of the wasp. This confirms previous observations on the apparently erratic distribution of

such substances in different species of insects (Bisset *et al.* 1960) and coelenterates (Mathias, Ross & Schachter, 1960).

The presence of ACh in concentrations up to 50 mg/g in dry hornet venom sacs and of a substance resembling DMAC, in concentrations of 1–2 mg/g, in the prothoracic (defensive) gland of the Garden Tiger Moth (*A. Caja*) (Bisset *et al.* 1960), suggests that ACh and other choline esters

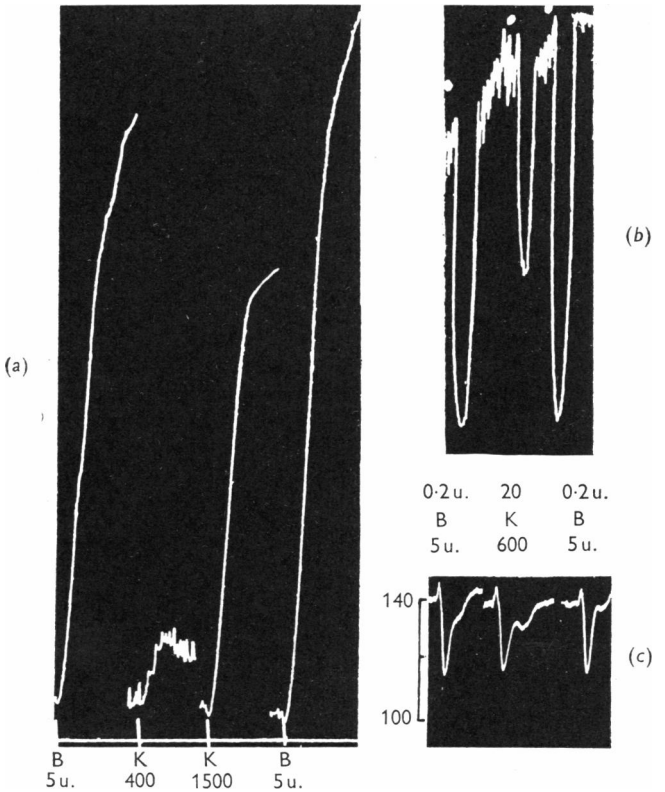

Fig. 8. Similar pharmacological actions of bradykinin and hornet kinin. (*a*) Guinea-pig ileum; (*b*) rat duodenum; (*c*) rabbit arterial B.P. B, bradykinin (units); K, hornet kinin (μg).

may be widespread in insects. The recent demonstration that the hypertrophied silk gland of the caterpillar (and cocoon) of *A. caja* and the abdominal tissue of the adult moth, contain large amounts of ACh (Morley & Schachter, 1961; Gill, Parsons & Paton, 1961) supports this view. It is possible that not only ACh, but also other choline esters, may have functions in nature other than the established one as a neuro-transmitter substance. Evidence suggesting a non-nervous metabolism of ACh in mammalian intestine has indeed been presented by Feldberg & Lin (1950).

The significance of ACh in a non-innervated organ such as human placenta (Chang & Gaddum, 1933) or of PrCh in ox spleen (Banister *et al.* 1953) has never been explained. The existence in nature of active choline esters other than ACh also underlines the need for specific identification of substances in tissues which show the general biological properties of ACh.

It has recently been shown that whole heads of various insects (flies, beetles and cockroaches) contain a substance resembling ACh in concentrations of 10–40 μg/g (Lewis & Smallman, 1956). It cannot be assumed in such experiments that the ACh is present only in the nervous tissue. The present experiments and those of Morley & Schachter (1961) demonstrate that ACh may be present in high concentrations in non-nervous tissue, and emphasizes the need for specific location of this substance.

The presence of a new kinin in hornet venom, closely resembling, but different from bradykinin (or kallidin) and wasp kinin, demonstrates that this type of pharmacologically active molecule, like choline esters and catecholamines, exists with some variations in animal tissues. It still remains to be seen whether these kinins will also be of physiological importance.

SUMMARY

1. ACh, 5-HT and histamine were identified in hornet venom sacs by parallel bioassay, paper chromatography and other chemical tests.

2. The ACh concentrations varied from 18–50 mg/g dry venom sac (mean 37·8); 5-HT and histamine were present in approximately one half the concentration of ACh.

3. A new kinin was demonstrated in hornet venom. This differs from wasp kinin in being resistant to inactivation by trypsin, in its behaviour on paper chromatograms, and in its relatively weaker action on the isolated guinea-pig ileum. It resembles bradykinin except that it is approximately one tenth relatively as active on the guinea-pig ileum as on other preparations.

4. The possible significance of ACh and other choline esters in nature is discussed.

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